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(54) Pre-storage filtration of platelets

Filtration von Blutplättchen vor der Lagerung

Filtration à pré-stockage des plaquettes

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(56) References cited:

EP-A- 370 584
WO-A-89/04639
WO-A-91/04088
US-A- 4 280 497
US-A- 4 596 657
US-A- 4 855 063
US-A- 4 880 548

- **VOX SANGUINIS, vol. 44, 1983, Basel G. SIRCHIA et al. "Prepara- tion of leukocyte-free platelets for Transfusion by Filtration through cotton wool" pages 115-120**
- **TRANSFUSION, vol. 29, no. 5, June 1989, Philadelphia T.S. KICKLER et al. "Deple- tion of white cells from platelet concentrates with a new adsorption filter" pages 411-414**

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Description

Background of the Invention

Field: This disclosure is concerned generally with the filtration of blood products and specifically with the filtration of white blood cells from platelets.

Prior Art: The advantages of removing white blood cells (WBCs) from blood components such as red blood cells (RBCs) and platelets are known. See, for example, U.S. Patent 4,596,657 to Wisdom (removal of WBCs from RBCs). The removal of WBCs from platelets is disclosed in U.S. Patent 4,596,190 to Kuhlemann et al., in EP-A1-0 370 589, and in articles by Sirchia, G., et al., Vox Sang. 44:115 - 120, 1983, and Kickler, T.S. et al., Transfusion 29:411 - 414, 1989.

Existing methods remove WBCs from the final form of platelet concentrates (PC) or pools of such concentrates. See the Kuhlemann et al. patent which describes a platelet pooling bag designed for this purpose.

EP-A1-0 370 589 is concerned with a filter for removing WBCs from PCs. Sirchia et al., Vox Sang. 44:115-120, 1983, describe the preparation of a PC which is then filtered to remove WBCs and RBCs including the step of preparing platelet rich plasma from human whole blood and using blood bags. The authers do not mention the use of a closed system. The present invention, however, is concerned with the removal of substantially all contaminating WBCs from platelet rich plasma (instead of PC) by means of a closed blood bag system.

Kickler et al. disclose filtration of a PC at the bedside, just before infusion into a patient. Before these existing methods can be used, however, the PC must be available. Unfortunately, the collection and initial processing of whole blood from a donor may result in a delay in the preparation of PCs.

In current practice at least two centrifugation steps are used to make a PC from whole blood. In the first step whole blood is collected in a blood bag and then centrifuged to form a lower, dense portion of RBCs and an upper, less dense portion of plasma which is rich in platelets and known as platelet rich plasma (PRP). The upper PRP is then expressed from the bag into a second bag which is centrifuged to form a lower, denser platelet pellet and an upper, less dense plasma portion known as platelet-poor plasma (PPP). The upper PPP is then expressed from the second bag for use in preparing various plasma products (e.g. albumin, immunoglobulins, coagulation factors and the like) leaving behind the plasma pellet or PC.

Even after the PC is made, however, it may be stored for some time (e.g., up to 5 days) before it is filtered to remove WBCs.

The presence of WBCs in a stored PC is thought to result in WBC degradation products that can adversely affect the platelets and their environment. Unfortunately, in conventional blood banking processes, there have been no available means for pre-storage removal of WBCs from platelets in a closed system. Thus, PCs, if filtered at all, have commonly been filtered just prior to infusion into the patient.

We have found that it is now possible to remove substantially all WBC's from a platelet product prior to platelet storage. This is done by filtering platelet rich plasma (PRP), soon after it is formed from centrifuged whole blood, using the novel closed system described below. The Filtration of WBCs from the PRP occurs within 8 hours of whole blood collection from a donor.

SUMMARY OF THE INVENTION

Our method of preparing platelets that are substantially free of WBCs requires the removal of WBCs from a platelet rich plasma (PRP) prior to forming a platelet concentrate (PC) and prior to any extended PC storage (e.g. 5 days).

According to the invention, there is provided a method of preparing platelets comprising the steps of (a) preparing platelet rich plasma from human whole blood; (b) passing the platelet rich plasma through a filter under conditions sufficient to remove substantially all white blood cells from the plasma; and (c) adding a platelet storage solution to the filtered platelets, said steps occurring in a closed blood bag system, with steps (a) to (b) occurring within eight hours of obtaining whole blood from a human donor. All of the steps are accomplished in a closed system. Examples of "closed" blood bag systems are well known. See, for example, U.S. Pat. No. 4,586,928 to Barnes et al.

According to the invention, there is also provided an apparatus as defined in claim 6.

The PRP is filtered as soon as possible or at least within about 8 hours of a whole blood donation. In one embodiment the closed system comprises a WBC filter disposed between a donor bag and at least two communicating satellite bags. The system includes a third satellite bag containing a platelet preservative (or additive) solution. Such solutions are well known. See, for example, U.S. Pat. 4,447,415 to G. Rock et al. and U.S. Pat. 4,695,460 to S. Holme. The third satellite bag (with additive solution) is a part of the claimed system and may be connected via sterile docking techniques to maintain a "closed" system. Examples of such sterile docking techniques are well known. See, for example, U.S. Pat. 4,507,119 and U.S. Pat. 4,443,215.

BRIEF DESCRIPTION OF THE FIGURE

The figure is a plan view of a preferred closed system for removal of WBCs from PRP soon after donation and centrifugation of whole blood.

SPECIFIC EMBODIMENTS

Our pre-storage filtration of platelets preferably uses a closed system such as that shown in the Figure. The Figure shows a donor bag 3 which may include a conventional anticoagulant solution such as CP2D, attached phlebotomy tubing 5 and needle 7 (illustrated by the arrow) connected via tubing 9 to an inlet port of WBC filter 11. Preferably, a valve such as frangible valve 13 (such as that shown in U.S. Pat. No. 4,586,928 to Barnes et al.) seals the contents of bag 3 until after bag 3 is centrifuged.

In closed communication with filter 11 via tubing 19 are at least two empty (MT) secondary (satellite) bags, 15a and 15b. A third satellite bag containing platelet additive solution (PAS) 17 is attached to the closed system via conventional blood bag tubing 21. Other bags may be added to the closed system for added uses.

In use, whole blood is collected into bag 3. The whole blood in bag 3 is then centrifuged using conventional methods to form an upper (less dense) platelet rich plasma (PRP) portion and a lower (more dense) packed red blood cell (RBC) portion. Then, valve 13 is opened and the PRP is expressed from bag 3 through filter 11 under conditions sufficient to remove WBCs and allow substantially all (more than 90%, preferably more than 99.5%) of the platelets to pass into one of the bags, 15a or 15b.

After the filtered PRP is collected into one of bags 15 via tubing 19, the donor bag 3 and filter 11 may be removed from the system by known means such as by cutting and sealing along tubing 19. The PRP in one of the bags, 15a, is then centrifuged to form a lower, denser platelet pellet and an upper, less dense platelet poor plasma (PPP) most of which can be expressed from the first satellite bag 15a to second satellite bag 15b via tubing 19a. Commonly, about 50 ml of residual plasma is left with the platelet pellet as a storage medium. At this point the second bag 15b containing the PPP can be removed from the system by cutting and sealing the connecting tubing 19a. This removed PPP can then be pooled with other PPP and used for other purposes such as plasma fractionation to produce useful blood components such as albumin, immunoglobulins, coagulation factors and the like.

At this point the platelet pellet remaining in bag 15a is resuspended in the residual plasma and is ready for use or storage for a period that can be up to five days.

To enhance storage, a platelet storage solution 17 from additional bag 21 can be added to bag 15. Examples of such solutions can be found in the above-cited patents to G. Rock et al and S. Holme. The disclosures of both patents are incorporated herein by reference to them.

Examples of preferred WBC filters that can be used in the above system are shown in U.S. Pat. No. 4,855,063 to Carmen et al. and U.S. Pat. No. 4,596,657 to Wisdom, the disclosures of which are also incorporated into this disclosure.

The tubings may be made from conventional polyvinyl chloride (PVC) blood tubing and the bags themselves are preferably made from plastic materials suited for their ultimate use. For example, in the case of platelet storage, the storage bags should have a high $0_2/C0_2$ gas transmissivity to control platelet pH. This can be accomplished using PVC film plasticized with trioctyltrimellitate (TOTM) as in U.S. Pat. No. 4,280,497 to Warner et al. or by using an ethylene vinyl acetate film. The donor bag may be made with the same plastic film or a different one more suitable for red blood cell storage (e.g. TOTM or, perhaps, DEHP plasticized PVC).

Specific examples and data are discussed below.

Examples

Three units of whole blood were collected into conventional donor blood bags. These bags were then centrifuged to separate PRP from red cells. Each unit of PRP was connected via conventional blood bag tubing to a WBC filter and expressed through the inline WBC filter (PL 100, available from Pall Corporation) into an attached blood bag similar to that of empty (MT) bag 15a of the Figure. The bag was made from a film of TOTM plasticized PVC of the type described in U.S. Pat. No. 4,280,497 to Warner et al. Before (and after) filtration leukocyte counts were:

Table I

Unit No.	Before No x 10^8	After No x 10^8	% Removed
272	0.47	0.005	98.9
273	1.81	0.009	99.5
274	1.52	0.005	99.7

After filtration, PRP units were processed via further centrifugation to PCs which were stored at 22°C on an agitator. In vitro data (mean values) in the table below show that pH was well maintained, platelets were consuming oxygen, morphology and hypotonic stress recovery were well maintained and there was no change in platelet number.

Table II

	Day 1	Day 4	Day 5	Day 6	Day 7
pH	7.472	7.532	7.497	7.436	7.244
PCO_2, mmHg	20.0	12.2	11.7	11.2	14.8
PO_2, mmHg	52.0	61.7	71.7	78.6	52.9
HCO_3, mM	17.8	12.1	11.0	9.3	7.4
% Discs, NAPSAC*	36.3	43.6	35.6	29.3	20.6
Hypotonic stress recovery, %	58.6	58.2	54.7	49.2	-
Platelets, No. x 10^{10}	5.1	5.2	5.2	5.2	5.1

*Non-Invasive Assessment of Platelet Shape and Function (NAPSAC) machine described in more detail in U.S. Pat. No. 4,522,494 to R.F. Bonner and available from Beecher Medical, Silver Spring, MD. See also, U.S. Pat. 4,753,797 to Garcez which describes the use of NAPSAC.

As used herein, the substantial removal of all WBCs means that at least 95% of the original WBCs are removed. In very preferred embodiments, at least 99.5% of the original WBCs are removed from the PRP.

Substantially all original platelets means at least 90% of the original platelets remain after the filtration step to be recovered in the platelet storage bag attached to the filter (or less than 10% of the platelets remain in the WBC filter).

The system of the figure can be modified as follows for alternate applications for filtration after the platelet concentrate has been made:

1. The filter 11 can be placed in line 21 such that after the platelets are concentrated in bag 15a, and all the platelet poor plasma has been transferred to bag 15b, the PAS is then transferred through the filter into the platelet pellet. Following resuspension, the platelet concentrate is then transferred back through the filter into bag 17 where the WBC-poor platelet concentrate will be stored.

Claims

1. A method of preparing platelets comprising the steps of (a) preparing platelet rich plasma from human whole blood; (b) passing the platelet rich plasma through a filter under conditions sufficient to remove substantially all white blood cells from the plasma; and (c) adding a platelet storage solution to the filtered platelets, said steps occurring in a closed blood bag system, with steps (a) to (b) occurring within eight hours of obtaining whole blood from a human donor.

2. The method of claim 1 wherein the filtration step is under conditions that result in a loss of less than 10% of the original platelets in the platelet rich plasma.

3. The method of claim 1 wherein the filtration is under conditions that remove at least 95% of the white blood cells.

4. The method of claim 1 wherein the filtration is under conditions that remove at least 99.5% of the white blood cells.

5. The method of claim 1 further comprising storing the filtered platelets for up to 5 days.

6. A closed system for the pre-storage filtration of platelets comprising a first blood bag (3) connected via a first tubing (9) to a white blood cell filter (11) which is connected via a second tubing (19) to a platelet storage bag (15a/15b), and a bag containing a platelet storage solution (17) which is also connected via tubing (21) to the platelet storage bag (15).

7. The system of claim 6 wherein the platelet storage bag (15) is made from plastic film comprising either polyvinyl chloride plasticized with trioctyltrimellitate or ethylene vinyl acetate.

Patentansprüche

1. Verfahren zum Bereitstellen von Plättchen, umfassend die Schritte

   a) Herstellen eines an Plättchen reichen Plasmas aus menschlichem Vollblut;
   b) Durchleiten des an Plättchen reichen Plasmas durch einen Filter unter Bedingungen, die ausreichen, um im wesentlichen alle weißen Blutkörperchen aus dem Plasma zu entfernen; und
   c) Zugeben einer Plättchenlagerungslösung zu den gefilterten Plättchen,

   wobei die Schritte in einem geschlossenen Blutbeutelsystem ablaufen, wobei die Schritte a) bis b) innerhalb von 8 Stunden nach Erhalt des Vollbluts von einem menschlichen Spender ablaufen.

2. Verfahren nach Anspruch 1, worin der Filtrationsschritt unter Bedingungen durchgeführt wird, die in einem Verlust von weniger als 10 % der ursprünglichen Plättchen in dem an Plättchen reichen Plasma resultieren.

3. Verfahren nach Anspruch 1, worin die Filtration unter Bedingungen durchgeführt wird, so daß mindestens 95 % der weißen Blutkörperchen entfernt werden.

4. Verfahren nach Anspruch 1, worin die Filtration unter Bedingungen durchgeführt wird, so daß mindestens 99,5 % der weißen Blutkörperchen entfernt werden.

5. Verfahren nach Anspruch 1, welches ferner das Lagern der gefilterten Plättchen während bis zu fünf Tagen umfaßt.

6. Geschlossenes System für die Filtration von Plättchen vor deren Lagerung, umfassend einen ersten Blutbeutel (3), welcher über eine erste Leitung (9) mit einem Filter für weiße Blutkörperchen (11) verbunden ist, welches über eine zweite Leitung (19) mit einem Plättchenlagerbeutel (15a/15b) verbunden ist, und mit einem Beutel, welcher eine Plättchenlagerungslösung (17) enthält, welcher ebenfalls mit einer Leitung (21) mit dem Plättchenlagerbeutel (15) verbunden ist.

7. System nach Anspruch 6, worin der Plättchenlagerbeutel (15) aus einer Kunststoffolie hergestellt ist, welche entweder Polyvinylchlorid, plastifiziert mit Trioctyltrimellitat, oder Ethylenvinylacetat umfaßt.

Revendications

1. Procédé de préparation de plaquettes, comprenant les étapes consistant à (a) préparer du plasma riche en plaquettes à partir de sang total humain ; (b) faire passer le plasma riche en plaquettes sur un filtre dans des conditions suffisantes pour séparer sensiblement tous les globules blancs du plasma ; et (c) ajouter une solution de conservation des plaquettes aux plaquettes filtrées, lesdites étapes étant opérées dans un système de poches de sang en circuit fermé, les étapes (a) et (b) étant mises en oeuvre dans les 8 h après l'obtention du sang total d'un donneur humain.

2. Procédé selon la revendication 1, où l'étape de filtration s'effectue dans des conditions qui aboutissent à la perte de moins de 10 % des plaquettes d'origine dans le plasma riche en plaquettes.

3. Procédé selon la revendication 1, où la filtration s'effectue dans des conditions qui séparent au moins 95 % des globules blancs.

4. Procédé selon la revendication 1, où la filtration s'effectue dans des conditions qui séparent au moins 99,5 % des globules blancs.

5. Procédé selon la revendication 1, comprenant en outre la conservation des plaquettes filtrées pendant un maximum de 5 jours.

6. Système en circuit fermé destiné à la filtration avant conservation de plaquettes, comprenant une première poche de sang (3) reliée par un premier tube (9) à un filtre de globules blancs (11) qui est connecté par un deuxième tube (19) à une poche de conservation des plaquettes (15a/15b) et une poche contenant une solution de conservation des plaquettes (17) qui est également reliée par un tube (21) à la poche de conservation des plaquettes (15).

7. Système selon la revendication 6, où la poche de conservation des plaquettes (15) est en un film plastique comprenant soit du chlorure de polyvinyle plastifié par du trimellitate de trioctyle, soit de l'éthylène-acétate de vinyle.

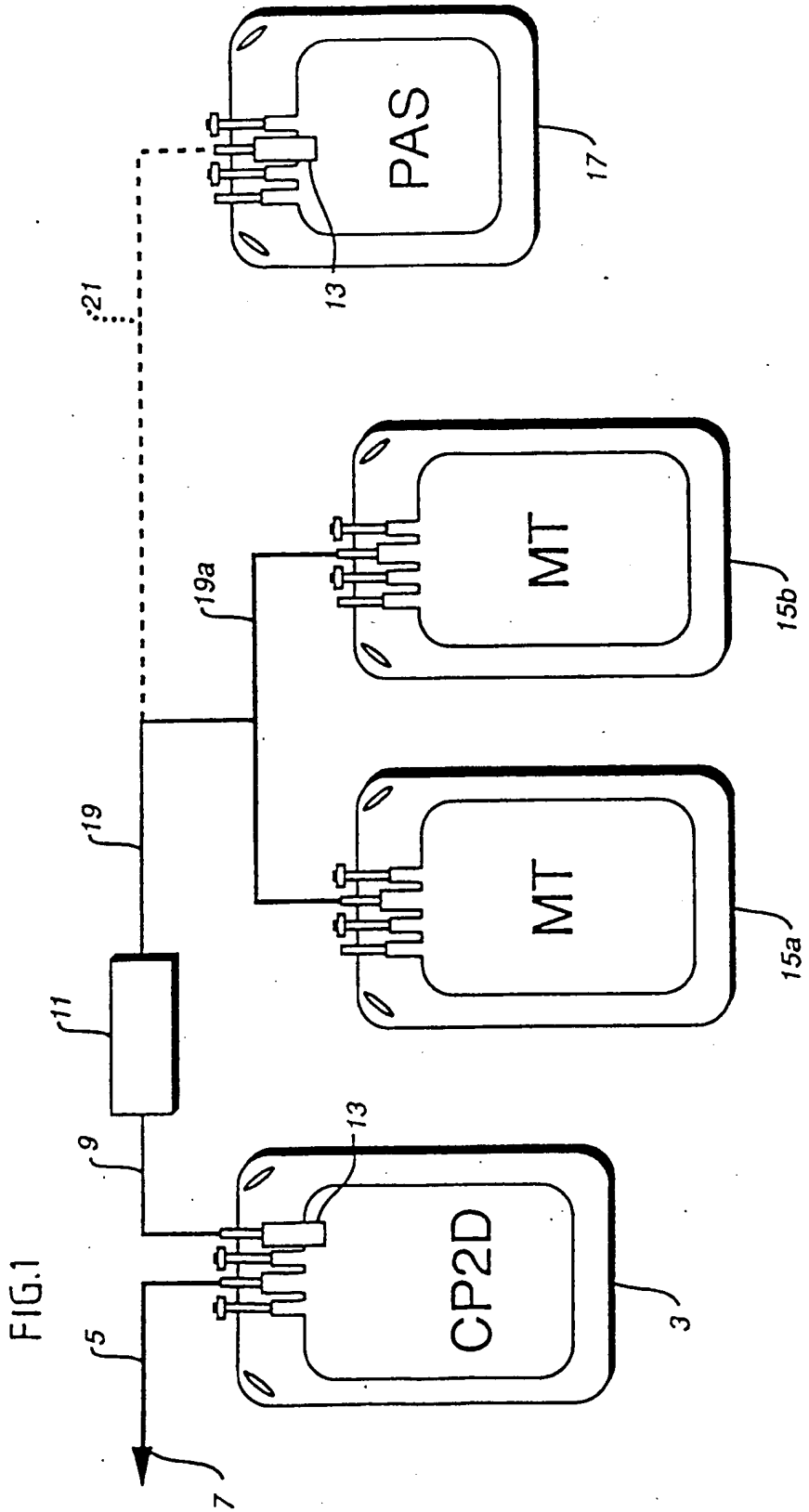

FIG.1